NOTES

CELLULAR STRUCTURE OF LARGE SPIRILLA

K. A. BISSET AND PHYLLIS PEASE

Department of Bacteriology, University of Birmingham, Birmingham, England

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Evidence from many different sources, and founded upon considerations of cytochemistry, flagellar development, and observational experiment, suggests that the main growth of unicellular bacteria is at the poles. This evidence is discussed more fully by Bisset and Pease (J. Gen. Microbiol., **16,** 382–384, 1957). In multicellular bacteria, conditions are more complex, and the poles of all constituent cells are possible areas of growth.

A recent paper by Williams (J. Bacteriol., **78,** 374–377, 1959) states that the main growth of a "unicellular" spirillum occurred in the central area; accordingly, this author takes issue with the supporters of the theory of polar growth.

In our experience, large spirilla are not unicellular, although it is not possible (as in the case of most other bacteria) to discern their structure by ordinary methods. Cross-walls can be demonstrated by appropriate techniques (figure 1), and may occasionally be visible in partially disrupted organisms by electron microscopy (figure 2).

We consider that a spirillum of the configuration illustrated by Williams would normally possess at least one developing cross-wall centrally; thus the active growth of the organism at this point (i. e., at the coincidence of two developing poles of the component cells), is absolutely in accordance with the suggestion that bacterial growth occurs mainly at the poles of the cells.

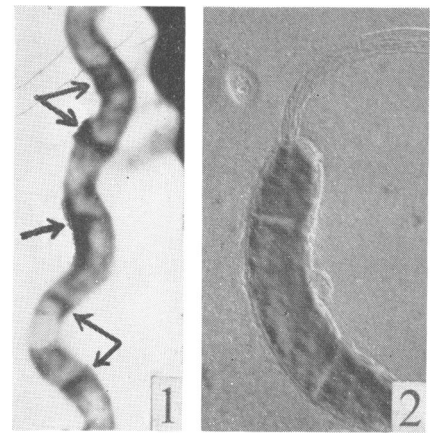

Figure 1. Spirillum sp.; mordant, 10 per cent phosphomolybdic acid and 0.3 per cent aqueous iodine; stain, 1 per cent methyl green. Arrows indicate cross-walls. (×3000)

Figure 2. Spirillum undula; electron micrograph, gold-shadowed, showing cross-walls. (×7500)

PHAGE T1-RESISTANT MUTANTS OF *ESCHERICHIA COLI*

ROGER WEINBERG[1]

Department of Medical Microbiology, University of Southern California, and Los Angeles County General Hospital,[2] Los Angeles, California

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Experiments were performed to obtain information concerning the genetic nature of the simultaneous mutation to phage T1 resistance and requirement for tryptophan (T1, try$^-$) in *Escherichia coli* (Anderson, Proc. Natl. Acad. Sci. U. S., **32,** 120–128, 1946; Skaar and Davidson, Cold Spring Harbor Symposia Quant. Biol., **18,** 120–121, 1956; Yanofsky and Lennox, Virology, **8,** 425–447, 1959). Phages used were P1 *kc* (Lennox, Virology, **1,** 190–206) and T1 (described in

[1] U. S. Public Health Service Postdoctoral Fellow CF-6931-C. Present address: Department of Biological Sciences, University of Pittsburgh, Pittsburgh 13, Pennsylvania.

[2] This work was initiated at the California Institute of Technology, Pasadena, California.

Adams, *Bacteriophages*, Interscience Publishers, New York, 1959). Bacteria used were a nonlysogenic Hfr Hayes derivative of *E. coli* strain K-12, (Wollman *et al.*, Cold Spring Harbor Symposia Quant. Biol., **21,** 141–162, 1956), referred to as strain K-12, *E. coli* strain C, referred to as strain C, and a derivative of strain K-12 containing the T1,try$^+$ locus (ability to synthesize tryptophan and sensitivity to T1) of strain C, referred to as K-12(C), which was obtained by transducing (by means of phage P1) first the T1,try$^-$ locus from strain C-5 of Bertani and Six (Virology, **6,** 357–381, 1959) into strain K-12, selecting for T1 resistance, and then the T1,try$^+$ locus from strain C into such a transductant, selecting for tryptophan independence.

Transductions with phage P1 were carried out essentially as described by Lennox (Virology, **1,** 190–206, 1955). Since P1 grown on strain C did not grow well on strain K-12, characters to be transduced into strain K-12 were first transferred by transduction from strain C to a hybrid between strain C and strain K-12 obtained in bacterial crosses, and then from the hybrid to strain K-12. Bacterial crosses were made using techniques described by Weinberg (J. Bacteriol. **79,** 558–563, 1960).

For isolation of T1-resistant mutants, single colonies of T1-sensitive strains were picked and streaked onto plates previously coated with T1. Less than half of such streaks gave any mutants at all. From each streak which gave rise to nonmucoid T1-resistant mutants, one such mutant was picked and streaked on a minimal agar plate containing phage T5 and tryptophan crystals on one side of the plate, and classified, after incubation, as to T5 resistance and tryptophan requirement. Rare mutants gave no growth on these plates. It was not investigated, however, whether these were caused by faulty technique or by requirements for factors other than tryptophan.

The distribution of mutant types obtained from strain K-12 (C) resembles that of the mutants from strain K-12 much more than that of the mutants from strain C (table 1). Since in strain K-12 (C) a small chromosomal region comprising the locus T1,try originated from strain C (by transduction), it appears that the high mutability to the mucoid, T1-resistant type is not controlled by factors located in such a region of the chromosome.

Of the T1,try$^-$ mutants obtained from K-12, 21 were crossed to two T1-sensitive tryptophan-requiring mutants obtained from Dr. C. Yanofsky, td-3 and T-4 (Yanofsky, J. Biol. Chem., **224,** 783–791, 1957), made F$^-$ by the acridine method (Hirota *et al.*, Nature, **180,** 655, 1957), selecting for tryptophan independence in the crosses. Only two gave recombinants. These two were then crossed to each other (for this purpose the two T1,try$^-$ markers were first crossed into K-12 F$^-$S^r (streptomycin-resistant) strains, so that Hfr × F$^-$ crosses could be made), but no tryptophan-independent recombinants were obtained.

TABLE 1

Recovery of various types of independently isolated, spontaneous T1-resistant mutants of Escherichia coli

Type of T1-Resistant Mutant	Per Cent of Mutant types Obtained from Strain		
	C	K-12	K-12(C)
Mucoid	2	45	45
Nonmucoid:			
Prototrophic, resistant to phage T5	70	27	44
Prototrophic, sensitive to phage T5	18	26	9
Requiring tryptophan, sensitive to phage T5	12	2	2
Requiring tryptophan, resistant to phage T5	0	0	0
Total mutants scored	53	451	110

None of the 21 T1,try$^-$ mutants gave rise to tryptophan independent revertants when approximately 10^{10} cells were spread on minimal agar. Thus the T1,try$^-$ mutation is probably a deletion in strain K-12 (Yanofsky and Lennox, Virology **8,** 425–447, 1959) as it seems to be in strain B (Skaar and Davidson, Cold Spring Harbor Symposia Quant. Biol., **18,** 120–121, 1956).

The results of Weinberg (J. Bacteriol., **79,** 558–563, 1960) indicate that the T1,try$^-$ marker is in the same order on the genetic chromosome as is the Try$^-$ mutation, unconnected with T1 resistance, used by Jacob and Wollman (Ann. inst. Pasteur, **95,** 497–519, 1958), as one would expect from Skaar and Davidson's, and Yanofsky and Lennox's findings.

Three T1-resistant, T5-sensitive, tryptophan-independent mutants isolated independently from strain K-12 were crossed to one of the K-12 F$^-$ T1,try$^-$ S^r strains mentioned above, and

tryptophan-independent recombinants were selected in the presence of streptomycin. The 19 recombinants tested from each of the three crosses were resistant to T1. When strain K-12 was used as the male, selecting in the same way, the 19 recombinants tested were T1,try$^+$. This indicates close linkage between the T1 locus and the T1,try locus in strain K-12, giving further support to the idea that the T1,try$^-$ mutation in K-12 is a simultaneous deletion of the T1 locus, and of some or all of the loci controlling tryptophan synthesis.

INDIGENOUS MARINE BACTERIOPHAGES[1]

R. SPENCER

Humber Laboratory, Department of Scientific and Industrial Research, Hull, England

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The presence in sea water beyond the littoral zone of bacteriophages active against marine bacteria has not been conclusively demonstrated despite their possible importance in marine microbiology. Although ZoBell (*Marine Microbiology*, 1946) isolated phages from sea water of the littoral zone, he was unsuccessful with water taken beyond. The phages isolated by Kriss and Rukina (Rept. U. S. S. R. Acad. Sci., **57,** 833, 1947) from the Black Sea were active against such typically terrestrial species as *Bacillus subtilis* and *Micrococcus albus* (Kriss *et al.*, Trans. biol. Sta. Sebastopol, **7,** 50, 1949, and Kriss, *personal communication*) and may thus have been adventitious, whereas the phage isolated by Smith and Krueger (J. Gen. Physiol., **38,** 161, 1954) against a marine vibrio was not strictly marine in origin. Consequently, attempts were made to isolate phages from sea water taken well beyond the littoral zone active against typically marine bacteria.

The sea water samples were taken from the North Sea, some 10 miles off Aberdeen, Scotland, and the marine bacteria consisted of two groups, strains of *Photobacterium phosphoreum* isolated from marine fish, and strains of several species isolated from a further sample of sea water. After preliminary experiments, two methods of isolation were used in parallel, one direct and one indirect, each with incubation at both 20 and 0 C.

The direct method consisted of layering on a nutrient agar base a mixture of 10 ml of 1.5 per cent nutrient agar, 10 ml of sea water previously membrane filtered to remove interfering bacteria, and 2 ml of a dense suspension of the appropriate bacterial culture. After incubation, the presence of any phage active against that particular culture was detectable by plaque formation. Samples (600 ml) of sea water at both 20 and 0 C were normally examined at a time against a total of 40 strains of bacteria.

In the indirect method, 300 ml of sea water were mixed with 100 ml of quadruple strength nutrient broth and 8-ml amounts of broth culture of four different bacterial strains. After incubation, the bacteria were removed by either filtration or by an adaption of the chloroform technique of Fredericq (Compt. rend. soc. biol., **144,** 295, 1950) involving carbon tetrachloride, and the bacteria-free preparation tested for lytic action against the appropriate bacterial strains by the above layer technique. Samples (3 L) of sea water at both 20 and 0 C were normally examined at a time, again with 40 strains of bacteria.

In all, 6 L of sea water were examined by the direct method and almost 40 L by the indirect method, and seven phages were isolated. Four of the phages were detected by the parallel direct and indirect methods at both 20 and 0 C; three were present in samples in a concentration of 1 to 5 particles per 10 ml, and one in a concentration of approximately 100 particles per 10 ml. The remaining three phages were detected by the indirect method.

One phage was active against a strain of *Photobacterium phosphoreum*, three against unidentified nonpigmented *Pseudomonas* species, two against an unidentified Flavobacterium, and one against an organism provisionally classified as a *Cytophaga* species.

[1] The work described in this paper was carried out as part of the program of the Department of Scientific and Industrial Research.